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Term:

L2 and 3'-5' exonuclease\$1	▲
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<u>L3</u>	L2 and 3'-5' exonuclease\$1	28	<u>L3</u>
<u>L2</u>	L1 and polymerase chain reaction\$1	2246	<u>L2</u>
<u>L1</u>	proteinase K	4466	<u>L1</u>

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- ☐ 1. [6399320](#). 01 Dec 98; 04 Jun 02. Modified DNA-polymerase from carboxydotherrmus hydrogenoformans and its use for coupled reverse transcription and [polymerase chain reaction](#). Markau; Ursula, et al. 435/15; 435/194 435/91.1 435/91.2 435/91.5 536/23.1 536/23.2. C12Q001/48 C12N009/12.
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- ☐ 2. [6365375](#). 16 Mar 99; 02 Apr 02. Method of primer-extension preamplification PCR. Dietmaier; Wolfgang, et al. 435/91.1; 435/6 435/91.2. C12P019/34 C12Q001/68.
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- ☐ 3. [6350580](#). 11 Oct 00; 26 Feb 02. Methods for detection of a target nucleic acid using a probe comprising secondary structure. Sorge; Joseph A.. 435/6; 435/91.1 435/91.2. C12Q001/68 C12P019/34.
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- ☐ 5. [6197557](#). 10 Sep 98; 06 Mar 01. Compositions and methods for analysis of nucleic acids. Makarov; Vladimir L., et al. 435/91.2; 435/6 536/23.1 536/24.3. C12P019/34 C12Q001/68 C07H021/02 C07H021/04.
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- ☐ 6. [6117635](#). 11 Apr 97; 12 Sep 00. Nucleic acid amplification oligonucleotides with molecular energy transfer labels and methods based thereon. Nazarenko; Irina A., et al. 435/6; 435/91.2 536/22.1 536/24.33 536/25.32. C12Q001/68 C12P019/34 C07H021/04 C07H021/00.
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- ☐ 7. [6117634](#). 06 Mar 97; 12 Sep 00. Nucleic acid sequencing and mapping. Langmore; John P., et al. 435/6; 435/91.2. C12Q001/68 C12P019/34.
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- ☐ 8. [6100078](#). 24 Feb 95; 08 Aug 00. Purified DNA polymerase from bacillus stearothermophilus ATCC 12980. Riggs; Michael Garth, et al. 435/194;. C12N009/12.
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- ☐ 9. [6090552](#). 11 Jul 97; 18 Jul 00. Nucleic acid amplification oligonucleotides with molecular energy transfer labels and methods based thereon. Nazarenko; Irina A., et al. 435/6; 435/91.2 536/24.3 536/24.32 536/24.33. C12Q001/68 C12P019/34 C07H021/04 C12N015/00.
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- ☐ 10. [6077664](#). 31 May 96; 20 Jun 00. Thermophilic DNA polymerases from Thermotoga neapolitana. Slater; Michael R., et al. 435/6; 435/183 435/320.1 435/91.1 435/91.2 530/350 536/23.2 536/23.7. C12Q001/68 C12P019/34 C07H021/04 C12N009/00.
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Term	Documents
3-5.DWPI,EPAB,JPAB,USPT.	84625
3-5S.DWPI,EPAB,JPAB,USPT.	7
EXONUCLEASE\$1	0
EXONUCLEASE.DWPI,EPAB,JPAB,USPT.	4896
EXONUCLEASED.DWPI,EPAB,JPAB,USPT.	8
EXONUCLEASES.DWPI,EPAB,JPAB,USPT.	1683
EXONUCLEASET.DWPI,EPAB,JPAB,USPT.	1
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L3: Entry 28 of 28

File: USPT

May 30, 1995

DOCUMENT-IDENTIFIER: US 5420029 A

TITLE: Mutated thermostable nucleic acid polymerase enzyme from thermotoga maritima

Brief Summary Paragraph Right (1):

The present invention relates to a purified, thermostable DNA polymerase purified from the hyperthermophilic eubactefia Thermotoga maritima and means for isolating and producing the enzyme. Thermostable DNA polymerases are useful in many recombinant DNA techniques, especially nucleic acid amplification by the polymerase chain reaction (PCR).

Brief Summary Paragraph Right (26):

The Tma thermostable DNA polymerase enzyme of the present invention satisfies the requirements for effective use in the amplification reaction known as the polymerase chain reaction or PCR. The Tma DNA polymerase enzyme does not become irreversibly denatured (inactivated) when subjected to the elevated temperatures for the time necessary to effect denaturation of double-stranded nucleic acids, a key step in the PCR process. Irreversible denaturation of an enzyme for purposes herein refers to permanent and complete loss of enzymatic activity.

Brief Summary Paragraph Right (69):

For portions of vectors or coding sequences that require sequence modifications, a variety of site-specific primer-directed mutagenesis methods are available. The polymerase chain reaction (PCR) can be used to perform site-specific mutagenesis. In another technique now standard in the art, a synthetic oligonucleotide encoding the desired mutation is used as a primer to direct synthesis of a complementary nucleic acid sequence of a single-stranded vector, such as pBS13+, that serves as a template. for construction of the extension product of the mutagenizing primer. The mutagenized DNA is transformed into a host bacterium, and cultures of the transformed bacteria are plated and identified. The identification of modified vectors may involve transfer of the DNA of selected transformants to a nitrocellulose filter or other membrane and the "lifts" hybridized with kinased synthetic primer at a temperature that permits hybridization of an exact match to the modified sequence but prevents hybridization with the original strand. Transformants that contain DNA that hybridizes with the probe are then cultured and serve as a reservoir of the modified DNA.

Brief Summary Paragraph Right (118):

Tma DNA polymerase is very useful in the diverse processes in which amplification of a nucleic acid sequence by the polymerase chain reaction is useful. The amplification method may be utilized to clone a particular nucleic acid sequence for insertion into a suitable expression vector, as described in U.S. Pat. No. 4,800, 159. The vector may be used to transform an appropriate host organism to produce the gene product of the sequence by standard methods of recombinant DNA technology. Such cloning may involve direct ligation into a vector using blunt-end ligation, or use of restriction enzymes to cleave at sites contained within the primers. Other processes suitable for Tma polymerase include those described in U.S. Pat. Nos. 4,683,195 and 4,683,202 and European Patent Publication Nos. 229,701; 237,362; and 258,017; these patents and publications are incorporated herein by reference. In addition, the present enzyme is useful in asymmetric PCR (see Gyllensten and Erlich, 1988, Proc. Natl. Acad. Sci. USA 85:7652-7656, incorporated herein by reference); inverse PCR (Ochman et al., 1988, Genetics 129:621, incorporated herein by reference); and for DNA sequencing (see Innis et al., 1988, Proc. Natl. Acad. Sci. USA 85:9436-9440, and McConlogue et al., 1988, Nuc. Acids Res. 16(20):9869). Tma polymerase is also believed to have reverse transcriptase activity; see PCT Patent Publication No. 91/09944, published Jul. 11, 1991, incorporated herein by reference.

Detailed Description Paragraph Right (76):

Both the pTma14 and pTma15 expression plasmids expressed at a high level a biologically active thermostable DNA polymerase of molecular weight of about 70 kDa; plasmid pTma15 expressed polymerase at a higher level than did pTma14. Based on similarities with E. coli Pol I Klenow fragment, such as conservation of amino acid sequence motifs in all three domains that are critical for 3'-5' exonuclease activity, distance from the amino terminus to the first domain critical for exonuclease activity, and length of the expressed protein, the shortened form (MET284) of Tma polymerase should possess 3'-5' exonuclease and proof-reading activity but lack 5'-3' exonuclease activity. However, initial SDS activity gel assays and solution assays for 3'-5' exonuclease activity suggested significant attenuation in the proof-reading activity of the polymerase expressed by E. coli host cells harboring plasmid pTma15.

Detailed Description Paragraph Right (90):

Before digestion, the PCR product was treated with 50 .mu.g/ml of Proteinase K in PCR reaction mix plus 0.5% SDS and 5 mM EDTA. After incubating for 30 minutes at 37.degree. C., the Proteinase K was heat inactivated at 68.degree. C. for 10 minutes. This procedure eliminated any Taq polymerase bound to the product that could inhibit subsequent restriction digests. The buffer was changed to a TE buffer, and the excess PCR primers were removed with a Centricon 100 microconcentrator.

Other Reference Publication (8):

Bernad et al., 1989, "A Conserved 3'-5' Exonuclease Active Site in Prokaryotic and Eukaryotic DNA Polymerases" Cell 59:219-228.